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ABSTRACT

The two-part set consists of a student handbook and a related teachers' handbook in allied health education for use at the tenth grade level. The student handbook consists of seven units which focus on the biology curriculum: (1) community water examination, (2) bacteriological examination of water, (3) the microscope, (4) microbes and man, (5) paper chromatography, (6) the transport system (part 1: heart rate and blood pressure, and part 2: examination of the blood), and (7) individual study--a report on a communicable or hereditary disease. Each unit offers learning activities and exercises which center on laboratory work in order to assist students in obtaining knowledge and skills related to allied health occupations. The teachers' handbook provides suggestions for activities to assist teachers in the instruction of five of the above units: (1) the microscope, (2) microbes and man, (3) paper chromatography, (4) transport system, and (5) examination of water. (EC)

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ALLIED HEALTH FIELD

TENTH GRADE

INTRODUCTION TO ALLIED HEALTH AND THE HEALTH CARE TEAM

Operation TACT

Curriculum

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ALLIED HEALTH FIELD

10TH GRADE CURRICULUM

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INTRODUCTION

re: A brief description of the rationale behind the Allied Health program as it pertains to the tenth grade Biology curriculum.

Biology, as defined, deals with life. It is this life, human life, and its relationship to the biological concepts studied, that lead us directly into the realm of Allied Health.

Each of the academic biological units will be put into a meaningful perspective as it relates to the health and welfare of mankind. The classroom tools and techniques will be compared to their counterparts in the health fields. Demonstrations of these tools and techniques will be carried out by the classroom instructors, visiting resource people, and students. At all times the basic concept will be stressed and the students' rudimentary experiences extended into the "real world".

It is by this means that we hope to generate the enthusiasm and interest which will enable the student to recognize the meaning and scope of community health.

Certain areas of the allied health program lend themselves more readily for inclusion in the tenth grade biology curriculum than others. Although just about all of the areas covered under the natural sciences can conceivably be projected into the area of health and welfare, it is our intention to include only those areas which we consider most conveniently applicable.

The units in Allied Health must not appear as representing a separate unit unto themselves. They must serve as a bridge which serves to link the purely academic with the work-a-day world.

The teaching of academic subjects without relating that knowledge to actual job tasks and skills may take on an aspect of irrelevancy to the student. In the same respect, teaching only job tasks and skills may not be sufficient to

prepare and motivate the student to seek further education with an eye toward better job opportunities.

The Allied Health curriculum will be concerned with the following seven areas of involvement:

Community Water Examination

Bacteriological Examination of Water

The Microscope (a diagnostic tool)

Microbes and Man

Paper Chromatography (an Analytical Tool)

The Transport System

Part I: Heart Rate and Blood Pressure

Part II: Examination of the Blood

Individual Study - A Report on a Communicable or Hereditary Disease

Each of the above units will be approached in a lab-oriented manner. The student will become an active practitioner in an exercise designed to capture the flavor of one or more of the areas concerned with community health. The lab exercises will, as closely as possible, approximate those activities which are performed in the field. He will, in effect, play the role of lab technician, pathologist, bacteriologist, dietitian, physical therapist, clinical nurse, lab technologist, etc.

It is this point that he must be made aware of these supportive areas, the specific tasks involved, type and period of training, job market, growth potential, salary, benefits, etc.

Much of the above information will come from those people who are at present fulfilling the various Allied roles in the community. These visits will be complemented by career pamphlets, library visits, films, film strips, tapes, models, closed circuit TV, role playing, medical forms, field trips, articles, and other forms of related activity.

No matter how neatly and completely these packages are tied together, it is still the individual class instructor who is responsible for their effectiveness. It is he who must be able to convey to the student the relatedness of their academic training in Biology to the opportunities related to Allied Health in their community.

Each Allied Health unit will be more or less in the manner outlined below:

A. Introduction
    1. background material
        a. significance
B. Behavioral Objectives
C. Lab Exercise
    1. objectives
    2. materials and equipment
    3. procedure
D. Review questions or problems
E. Unit Test
F. Resource information
    1. who, what, where, when
        a. How to utilize
G. Bibliography
H. Supplementary activities

UNIT I

COMMUNITY WATER EXAMINATION

COMMUNITY WATER EXAMINATION

PROLOGUE

In many communities direct threats to human health arrive through the faucet as well as through the air. The drinking water that flows through the tap in some localities has already passed through seven or eight people. Graffiti in public washrooms in several towns along the upper Mississippi River reads: "Flush the toilet, they need the water in St. Louis". When the water reaches St. Louis it is chlorinated and filtered, and therefore should be safe to drink. Unfortunately, the water in many cities is unsafe to drink. Infectious hepatitis is spreading at an alarming rate in the United States, and a major suspect for the route of transmission is the "toilet-to-mouth" principle of many water systems not made safe by chlorination.

Besides hepatitis, the disease producing organsims which most frequently invade our intestinal tract cause typhoid fever, dysentery, and cholera. Water can be perfectly clear in appearance, free from peculiar odors and taste, and yet contaminated. Obviously special procedures are necessary to determine its sanitary quality.

As the population of many communities grow, their sewage-treatment facilities, though once adequate, are quickly outgrown. Thus we need more federal controls and regulations as well as an increase in the efforts of sanitarians, engineers, and public health inspectors. Constant examination of our surface and sub-surface water must be carried out as a constant safe guard against water pollution.

It is the intention in this unit to expose you to some of the methods of water analysis which are performed in the field. It will be interesting to see what data we can collect from an investigation of the ponds, brooks, streams, gullies, lakes, and shore front of our community.

OBJECTIVES

The student will become familiar with and involved in some of the "standard methods" employed in the examination of water. By way of assigned reading, discussion, and laboratory activities, the student will gain a greater insight into and appreciation of one of our most precious commodities, water. It is hoped that the student will feel a sense of personal involvement by becoming a part of a community concern which is both timely and relevant.

* Property	Gram pos	Gram neg
Susceptibility to sulfonamide drugs and penicillin	Marked	Much less
Susceptibility to low surface tension	Marked	Much less
Susceptibility to lysis by complement	Slight	Marked
Digestion by trypsin or pepsin (dead cells)	Resistant	Susceptible
Cell wall digestible by lysozyme	Many species	After treatment of the cell wall
Dissolved by 1% NaOH	Resistant	Susceptible
Aromatic and S-containing amino acids in cell wall	None	Numerous
Ratio of RNA to DNA in the cell	8:1	Almost equal

*(After Frobisher - Fundamentals of Microbiology)

ODOR

Odors in water are caused by extremely small concentrations of volatile compounds. Some odorous materials are detectable when present in only a few parts per million (ppm), laboratory procedures are unsatisfactory for their measurement and reliance is placed on the sense of smell. The sense of smell varies with individuals and, therefore, the results will also vary somewhat. It is important that you attempt to do this test in an odor-free room with odor-free equipment. Sample should be at room temperature for odor test.

Sanitary significance

1. Some odors are produced when organic matter decomposes and are likely to be present in the surface waters due to the presence of organic matter from surface drainage. Intensity and offensiveness varies with the type of organic material, some being earthy, greasy and musty, while other are sewage-like.
2. Most of the objectionable odors in surface waters are caused by plankton (microscopic plants and animals), which liberate minute traces of volatile oils when their numbers are decreasing and the dead organisms are decomposing.
3. The use of chlorine for disinfection frequently destroys odor-producing substances, but in many cases, has also been known to accentuate specific types of odors.

TABLE I

Express the intensity of the odor by a numeral relating to the term which describes its quality as follows:

Numerical Value	Term	Definition
0	None	No detectable odor
1	Very faint	An odor that would not be detected ordinarily by the average consumer, but that could be detected by an experienced observer.
2	Faint	An odor that the consumer might detect if his attention were called to it.
3	Distinct	An odor that would be detected readily and might cause the water to be regarded

Table 1 (cont'd.)

		with disfavor.
4	Decided	An odor that would force itself upon the attention and that might make the water non-drinkable.
5	Very Strong	An odor of such intensity that it would be absolutely unfit to drink. (This would be an extreme case).

TABLE II

Odor Characteristics

Name of odor	Description -"such as odors of -"
Aromatic	Cloves, lavender, lemon
Balsamic (flowery)	Geranium, violets and vanilla
Chemical	Industrial wastes or chemical treatment
Chlorinous	Free chlorine, home disinfectants
Hydrocarbon	Oil refinery wastes, marsh gas
Disagreeable	Pronounced unpleasant odors
Septic	Stale sewage
Earthy	Damp Earth
Grassy	Crushed grass
Musty	Decomposing straw
Mouldy	Damp cellar
Vegetable	Root vegetables

Data Recording Sheet

COMMUNITY WATER STUDY

COLLECTION

Town:_____ Map Coordinate _____

Date:_____ time:_____ Water temp °C

surface_____

one foot_____

Physical layout:_____

_____

_____

_____

_____

_____

Collection depth: (check)

surface _____

six inches _____

one foot _____

two feet _____

other_____

Day:

Temp_____°C.

sunny_____

cloudy_____

other_____

Odor: (room temp.)

none_____

slight_____

strong_____

oily_____

fishy_____

sweet_____

other_____

Surface features:

calm _____

ripples_____

choppy_____

other_____

Wind:

calm_____

breezy_____

blowing_____

direction_____

*Taste: (room temp.)

salty_____

mettalic_____

other_____

_____

*Do not taste water if in doubt of sanitary condition

BACTERIAL COUNT

Standard Plate Count

sample number	no. of colonies counted	dilution factor	total bacteria per ml	date

remember no. of colonies counted x dilution factor = total bacteria per ml

Coliform Plate Count

sample number	no. of colonies counted	dilution factor	total bacteria per ml	date

Sample pH (acidity/alkalinity)

cold_____

room temp_____

UNIT II

BACTERIOLOGICAL EXAMINATION OF WATER

BACTERIOLOGICAL EXAMINATION OF WATER

Natural waters contain a wide variety of microorganisms. In fact, it is not unlikely that one might find representatives of each of the major categories of microorganisms in any one sample. The fact that these many and varied organisms are present is not alarming in itself. It is their overall numbers and types which are of sanitary significance.

It is our task at this point to actually count those invisible 'bugs' which are present in our collected sample.

Taking into account, the time element, equipment involved, and cost of materials, your instructor might wish to make this a two-fold problem. That is not only to count the organisms present but also to identify a particular group of possible harmful bacteria.

PROCEDURE A - Standard Plate Count

One of the routine procedures for determining the bacterial content of different sources is the plate-count technique. This procedure is based upon the assumption that each viable (living) cell will develop into a colony. The number of colonies on your plate reveals the number of organisms contained in your sample which were capable of growing under the specific conditions of incubation.

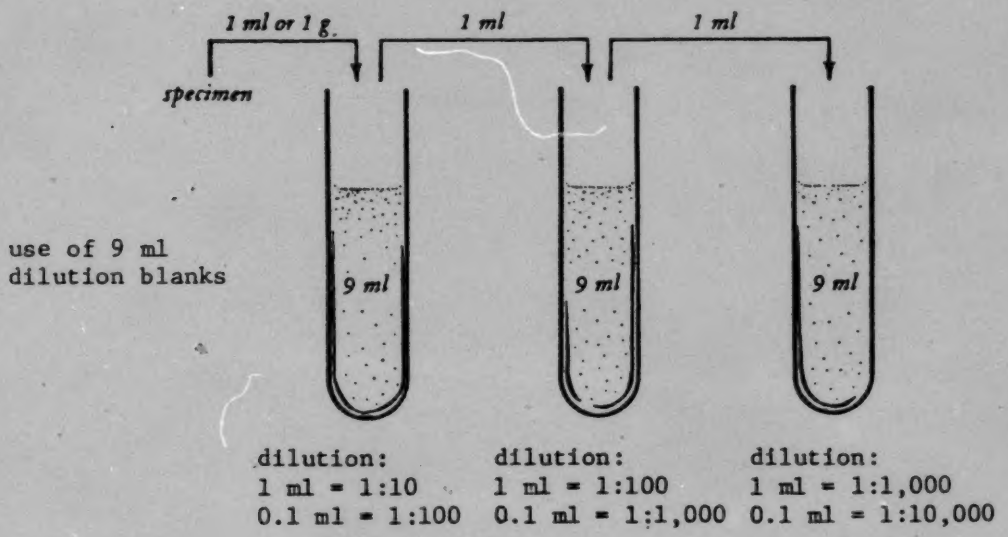
Procedure (cont'd)

a. dilution of specimen (if necessary)

The specimen is diluted in order that one of the final plates will have between 30 and 300 colonies. Numbers of colonies within this range give the most accurate approximation of the microbial population. Since the magnitude of the microbial population in the original specimen is not known beforehand, a range of dilutions must be prepared and plated to obtain one within the above colony range.

The initial dilution is usually prepared by placing 1 ml or 1 g into a 9- or 99- ml dilution blank.* This dilution is shaken vigorously to obtain uniform distribution of organisms. Further dilutions are made by pipetting measured portions (usually 1 ml) into additional dilution blanks. Each dilution must be thoroughly shaken before removing an amount (usually 1 ml) for subsequent dilution.

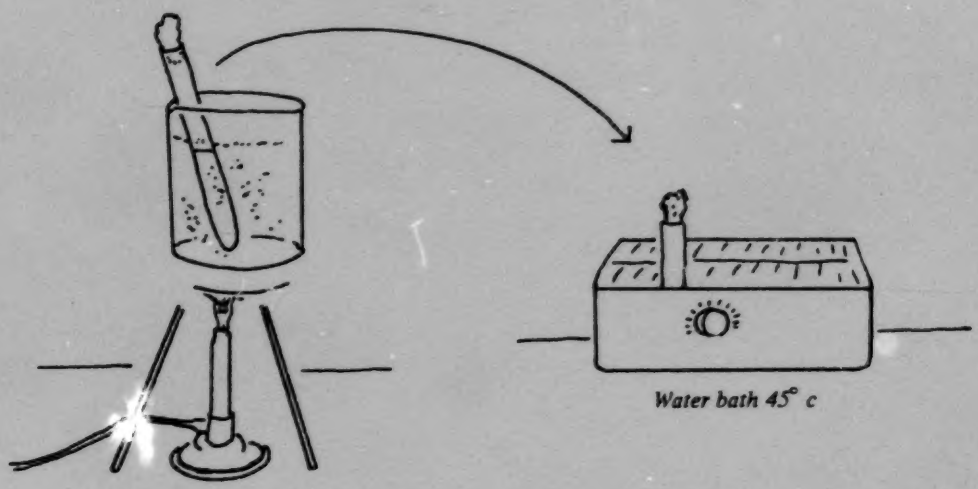
The procedure is illustrated diagrammatically below:



*Dilution blanks are tubes or bottles containing a known volume of sterile water. The 9- or 99-ml blank refers to the amount of sterile water in the container.

procedure (cont'd)

b. Place the provided nutrient agar tube into a water bath and melt the agar (100°c). After the agar has been melted place into a water bath (45°c) so that it will be ready when you need it. See below:

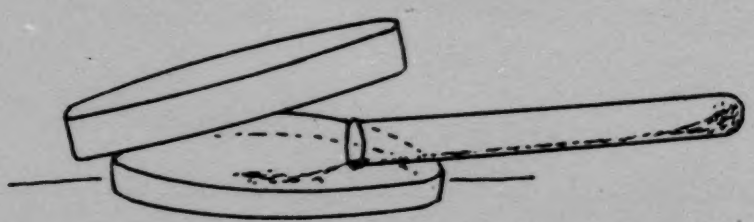


Water bath 45° c

Melt nutrient agar in water bath:

c. After preparing your sample for plating (by dilution), shake well and remove 1 ml and mix it with the melted agar (45°c). Mix the agar tube thoroughly by rotating between palms several times.

Quickly pour into bottom half of petri dish as illustrated. Allow the agar to solidify, invert, and place in incubation at 35°c. for 24-48 hrs.



d. After the prescribed period of incubation make an accurate count of the number of colonies by placing the plate on the Quebec colony counter. This instrument facilitates the counting process, since the colonies are illuminated, magnified, and seen against a ruled background.

e. The number of colonies counted on a plate, multiplied by the dilution factor equals tha plate count per milliter of the organism. For example, if 180 colonies were counted and the dilution was 1:1000, the calculation is:

180 x 1,000 = 180,000 - count/ml of water sample

f. Enter your data on the recording sheet.

FECAL POLLUTION

Introduction:

Pollution may be broadly defined as anything, either physical or chemical, that affects the natural conditions prevailing in either soil or water. The pollution of drinking water supplies with human feces has been recognized for many years as a public health problem rather than an ecological one. Only relatively recently has it become apparent that large amounts of sewage being emitted from crowded communities can pose a threat not only to the health of those individuals living in the community, but also to the ecological balance of the surrounding environment.

Since Escherichia coli, a coliform bacteria, is a microorganism whose natural habitat is the human animal colon, its presence in water is a clear indication that at some point that water has been polluted with either human or animal feces. The inference is made here that if E. coli is present, fecal pathogens might well be present also.

PROCEDURE B - Coliform Count

a. The procedure for obtaining a coliform count is the same as that previously described in Procedure A. (Standard Plate Count)

b. The only difference is that you will melt and pour a bacterial medium (agar tube) which is specific for the growth of coliform type bacteria. Other types of organisms will be killed by the stain in this media and only coliform types will grow.

*(thus coliforms being intestinal bacteria, if present in large numbers would indicate a polluted condition)

c. Incubate plate at 35°c for 24 hours.

d. Determine number of colonies and record.

Materials:

collected sample, sterile pipette, nutrient agar tubes (2), sterile dilution blanks (several 9ml & 99ml), *differential agar tubes (2), sterile petri dishes (2), constant temperature both, incubator, colony counter

*only if coliform count is to be performed

UNIT III

THE MICROSCOPE
(a diagnostic tool)

THE MICROSCOPE
(a diagnostic tool)

The microscope, ever since its discovery, has become more and more a necessity in the fields of science and medicine. The ability to extend mans sense of sight into the realm of the 'invisible' has added greatly to mans knowledge and understanding of both the physical and the biological universe.

From the simple hand lens, to the student microscope, and on to the electron microscope, man has been able to look into a world that he never even dreamed existed.

Most people tend to think of health services in terms of doctors, dentists, and nurses. It is true that this highly trained core of professionals is a key element of the health service work force, but they account for little more than one-third of the total health service personnel. Two-thirds of this service is provided for by supportive and specialized departments.

More and more of the diagnostic work is being performed by the clinical laboratories department. This department is responsible for testing in the six main fields of: bacteriology, biochemistry, histology(animal tissue), serology(tissue fluids), hematology(blood), and cytology(cells). It is the job of the various personnel in these fields to prepare and examine tissue in order to provide data on cause and progress of disease.

These clinical services are supervised by the Pathologist who is in charge of the various departments and personnel involved with the clinical laboratories.

Objective

This unit is designed to familiarize the student with the use of the microscope as a diagnostic tool. It is hoped that he will get the feel of discovery by microscopically examining tissue slides in search for a diseased condition or an infective organism.

He will work with some of the more familiar, obvious examples of normal and abnormal tissue in order to remain within his realm of experience.

But the concept and the activity will be closely related to the actual function in the clinical laboratory by the cytologist, pathologist, hematologist, etc.

Materials:

Microscope, prepared slides, normal and pathologic thssue.

Part A

Disease: Trichonosis (trick-o-no-sis)

Background:

This disease is caused by a minute roundworm known as the Trichina (Tra-kine-a) worm. Heavy infections may be severe or even fatal. This much dreaded parasite of man is usually obtained by eating insufficiently cooked pork. The worms become sexually mature in the human intestines and the female gives birth directly to larval worms. The worms are spread throughout the body by the circulatory system and then tend to burrow into the body muscles. It is at this point that the host (man) may experience fever, hard, painfully swollen muscles, anemia, swelling of various body parts, difficulty in swallowing and breathing, and death in about one third of the cases. It has been stated that from 12 to 20 percent of the people in the United States have, to some degree, been infected by this parasite.

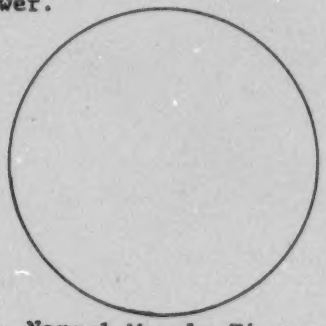
Task:

Comparison of Normal and Infected Skeletal Muscle

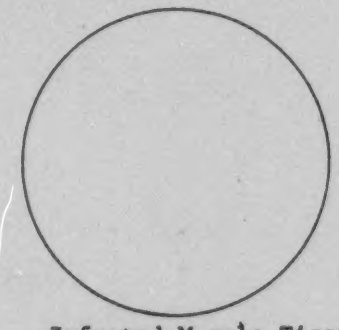
Procedure:

1. Obtain prepared slides of both normal and infected muscle tissue. Your instructor will familiarize you with the characteristices of healthy muscle tissue.

2. Observe both slides under low, medium, and high powers. Sketch a portion of both the normal and the infected tissue under high power.



Normal Muscle Tissue



Infected Muscle Tissue

Questions:

1. What sort of protective measures can be taken to prevent the spread of Trichina infection?

2. What body systems does this parasite invade once the infected pork is eaten?

3. What governmental agency (s) either federal or local acts to protect citizens against infected foods?

Part B

Disease: Sickle Cell Anemia

Background:

Sickle Cell is a hereditary disease effecting the red blood cells. More specifically, it effects the hemoglobin (heem-a-glow-bin) contained inside of the red blood cells. The basic function of the red blood cells and the hemoglobin is to carry oxygen to all the cells and tissues of the body so that they may function properly.

The disease tends to cause distortion of the red cells and rupturing of the cell membranes. This in turn cuts down on the oxygen carrying capacity of the cells, and thus leads to destruction of cells and tissues. Anemia, pain in muscles and joints, infection, blood clots, crippling and utimately death, results from this disease.

The disease strikes mainly those of the black race, mediterranians, and other ethnic groups who originated in areas of the world where malaria occurs. There is no known cure for this disease, but steps are being made which tend to soften the symptoms to a certain degree.

Task:

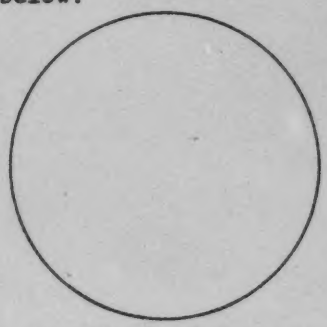
Compare healthy blood tissue with tissue taken from a victim of Sickle Cell.

Procedure:

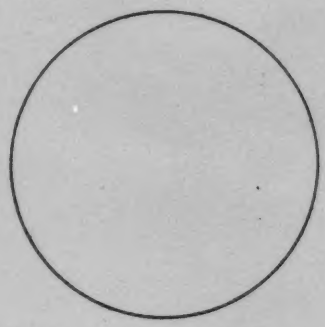
1. Obtain from your instructor a prepared slide of healthy blood and one showing the results of Sickle Cell.

2. Observe the normal blood smear under high power. Your instructor will describe what you should see in the field of vision and help you identify structure.

3. Now observe the Sickle Cell slide and sketch a portion of each below:



Normal Blood Smear



Sickle Cell Blood Smear

Questions:

1. What is the difference between the sickle cell trait and the sickle cell disease?

2. Define the term anemia.

3. To what local agency would you go if you wish to be tested for sickle cell?

4. What body system(s) is primarily effected by this dread disease?

5. What is the primary function of red blood cells in the body?

Part C

Disease: Hookworm

Background:

Another tiny roundworm which causes serious infection in man is the hookworm. This worm is largely confined to rural parts of our southeastern states, where millions of people are affected.

These minute worms develop in the soil and after reaching a certain stage they may infect man by burrowing through his skin. This entry is most easily accomplished in localities where people have the habit of going barefoot.

The hookworm goes on quite a journey through the body. It enters through the skin and works its way into the circulatory system where it is carried throughout the body. The worm is carried through several body organs by the blood and finally takes up temporary residence in the lungs. It burrows through the lung tissue and makes its way up the air tube (trachea) until it reaches the mouth cavity where it is swallowed and makes its way to the intestine. In the intestine, the adult worm attaches to the walls by sharp plate-like teeth and proceeds to dine on blood, lymph, and bits of mucus membrane. After mating the female releases thousands of eggs which pass out with the feces to continue the cycle.

The symptoms of the disease are widely known; anemia, laziness, and general lack of physical and mental energy. These conditions lead to a retardation of physical and mental development.

Task:

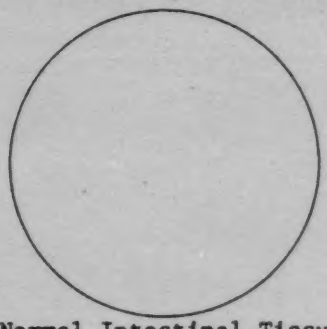
Comparison of normal healthy intestine tissue with tissue that has been invaded by the hookworm.

Procedure:

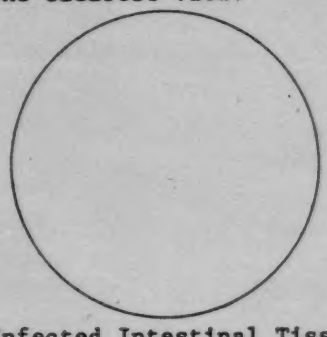
1. Obtain slides of normal and infected intestinal tissue.

2. Study the normal tissue and sketch a portion of it below.

3. Study the infected tissue, locate the worms and sketch below. Use the power which gives you the clearest view.



Normal Intestinal Tissue



Infected Intestinal Tissue

Questions:

1. List the body systems that the hookworm travels through after entering the body.

2. Relate at least one preventative measure which might be taken to eliminate the activity of the hookworm.

3. Is an "intermediate host" involved in the life cycle of the hookworm?

UNIT IV

MICROBES AND MAN

MICROBES AND MAN

The science of microbiology involves the study of organisms (microbes) so small that they cannot be seen with the naked eye. Rather they require the use of a powerful microscope in order to be observed.

Microorganisms are distributed throughout nature and interact with man and all other forms of life. They are present virtually everywhere, and their associations are essential to all forms of life. For example, certain bacteria are normal inhabitants of man's intestinal tract. These microorganisms benefit from this association, for they derive essential food materials from the host. They also benefit their host, for they manufacture and aid in the digestion of certain vitamins which are essential to the life of man.

Although microorganisms are distributed throughout nature and are generally beneficial and essential to life, there are certain organisms which are not beneficial. Rather, some are harmful to their respective hosts. These are the disease producing, or pathogenic, microorganisms.

The pathogenic organisms which may cause disease in man include members of both the plant and animal kingdom. The majority of pathogenic organisms belong to the plant kingdom, including the fungi, bacteria, and other simpler organisms. Although the study of microbes involves more than just bacteria, the following unit will be limited to only the bacteria.

It is the job of the hospital bacteriologist and his staff of technologists and technicians to track down and identify correctly the causative agent of infection. This may require many testing procedures. These tests involve a general knowledge of microorganisms and their mode of action.

In the following exercises you will be exposed to several of the basic techniques employed in the clinical laboratory when working with bacteria. Remember, these invisible organisms guard their secrets very jealously and only a very careful worker can pry these secrets from them.

1. You must familiarize yourself in advance with the exercise(s) to be performed.

2. Preliminary instructions and demonstrations will be given at the beginning of each exercise by the laboratory instructor. You should be prepared to ask any questions concerning the procedures to be followed and should thoroughly understand the purpose of the exercise.

3. Accurate and detailed results are to be recorded at the completion of each exercise. Use the tables and other spaces provided for recording results and answering questions.

MICROBES AND MAN

PERFORMANCE OBJECTIVES PARTS I, II, AND III

1. You must be able to identify the following pieces of equipment used in work with microbes; petri dish, culture tube, transfer loop, transfer needle, forceps, dropper bottle.

2. You should be able to display the proper techniques involved in organism transfer from: a) broth tube to broth tube; b) plate to broth tube; c) plate to plate (streak); d) tube to plate (pour plate); e) tube or plate to slide.

3. Be able to explain the significance of the Gram's stain.

4. Be able to define the terms: morphology, "fix"; sterile; colony; incubate; inoculate.

5. Be able to diagram if the name is given, or name if the diagram is given, the three main forms and the sub forms of bacterial cells.

6. In regard to the Gram's stain technique, be able to relate the purpose of the a) primary stain; b) mordant; c) counterstain.

7. Be able to relate at least two characteristics of the plating media agar which make it ideal for the study of bacterial organisms.

Laboratory Exercises Microbes and Man

Part I Materials

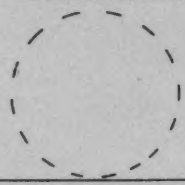
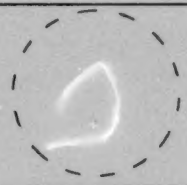
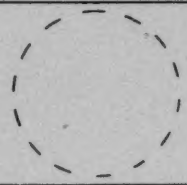
petri dish (cultures previously prepared)
culture tube
transfer loop
transfer needle
forceps
dropper bottle
staining solutions
methylene blue
carbol fuchsin
crystal violet
transfer needle or loop
microscope slides
oil (for oil immersion)

Part II
Cultures of B. subtilis and E. coli
staining reagents (crystal violet)
Gram's iodine
95% alcohol
safranin
clean slides
distilled H_2O
burner
absorbent paper discs

Part III
nutrient agar plate
slides
Gram stains
agar tube
sterile petri dish
transfer loop
variety of disinfectants
absorbent paper discs
forceps

FINDINGS: The Simple Stain

Make a drawing, and give a word description of the organisms found in each stained preparation. Describe the type of colony from which each smear was made.

	Methylene Blue	Carbol Fuchsin	Crystal Violet
Draw a few cells as observed in the microscopic field			
Word description of bacterial structure			
Description of colony from which slide was made			

LABORATORY EXERCISE

PART I.

THE SIMPLE STAIN

Introduction:

Unstained bacterial cells are nearly transparent when observed with the light microscope and hence are difficult to see. However, most bacteria can be readily stained with solutions of basic dyes.

You will "pick" some bacterial cells from the cultures you have prepared earlier. You should pick from several different colonies and employ each of the three stains available.

Materials:

Petri-dish cultures previously prepared; staining solutions: methylene blue, carbol fuchsin, crystal violet, transfer needle or loop.

Procedure: (read over completely before starting)

1. Wash and dry several microscope slides
2. Place a small, clean drop of water on the slide by using the transfer loop.
3. Select one of the culture dishes and, after flaming the transfer needle, remove a portion of a colony on the tip of the needle.
4. With a circular motion mix the bacterial cells into the water and spread the drop out to the approximate size of a dime. Flame the needle again.
5. Allow the smear to air-dry, then fix the smear by passing the slide through the flame several times.
6. Select one of the following stains and cover the smear for the designated time: crystal violet 20-60 seconds; methylene blue 1 to 2 min; carbol fuchsin 15 to 30 seconds.
7. After stain has set for desired period, remove excess stain by washing with a gentle stream of water, then blot (do not rub) dry with absorbent paper.
8. Examine the stained preparation under oil immersion.
9. Repeat these procedures using other colonies and different stains.
10. Complete the accompanying work-sheet.

LABORATORY EXERCISE

PART II.

THE GRAM STAIN

One of the most important and widely used procedures for characterizing bacteria is the gram stain. In general terms, bacteria are divided into two groups, based on whether they retain or lose the primary stain (crystal violet). Those organisms which retain the crystal violet (appear dark blue or violet) are designated gram-positive. Those organisms which lose the crystal violet and are subsequently stained (red) by the safranin counterstain and are designated gram-negative.

Such a separation into gram-positive and gram-negative categories is helpful in determining subsequent tests for eventual identification of a particular bacteria. It is also useful to the doctor for treatment of the patient, for certain antibiotics are generally effective against gram-positive bacteria, while gram-negative bacteria are not affected.

Materials:

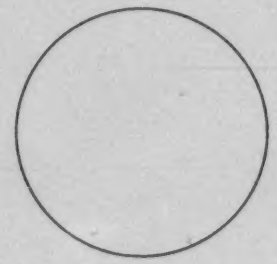
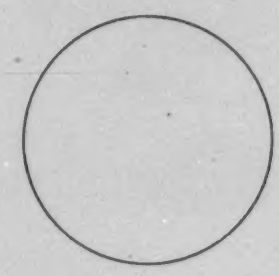
Cultures of B. subtilis and E. coli; staining reagents - crystal violet, Gram's iodine, 95% alcohol, safranin; clean slides; distilled H_2O.

Procedure:

1. Prepare smears of the B. subtilis and E. coli. Fix the smears over burner (slide should not be heated past the point where it feels very warm when applied to back of hand).
2. Flood the slides with crystal violet. Stain for 1 minute; then wash with water for a few seconds (until run-off is clear).
3. Apply Gram's iodine solution for 1 minute; wash with water and drain.
4. Decolorize with alcohol (95%) until free color has been washed off (approx. 30 sec.). Wash slide with water, and drain.
5. Counterstain smears for 30 sec. with safranin; wash, and blot dry.
6. Make microscopic examination of stained preparations.

RECORD

The Gram Stain

	B. subtilis	E. coli
drawing of a few cells under microscope		
magnification		
Gram stain positive or negative?		
color of cell		
description of cell morphology		

What is the advantage of a Gram stain over a simple strain?_____

_____

_____

_____

LABORATORY EXERCISE

PART III.

TRACKING AN UNKNOWN

Introduction:

Now that you have had an introduction to the world of the microbe, let's see you put some of your knowledge to work in the role of a microbe hunter. Your instructor has grown some bacteria in several culture dishes by allowing the decay of several familiar substances. It is your task to take a sample of the liquid from one of these culture dishes and subject it to several common tests in the hope of discovering some of the characteristics of the little beasts.

You will take a loop of this living liquid and make a streak plate which you will incubate for 24 hrs. You will then pick from one isolated colony which has grown and inoculate a tube of melted agar and incubate for 24 hrs. From this same colony you will perform a gram stain. After incubation for 24 hrs., the nutrient broth should appear turbid (cloudy). With this tube you will make a pour plate. When the pour plate hardens you will test to see the reaction of your organisms to several different disinfectants.

The following flow chart will give you quick summary of the steps involved:

Materials:

nutrient agar plate, slides, Gram stains, agar tube, sterile petri dish, transfer loop, variety of disinfectants, absorbent paper discs.

Procedures:

1. Sterilize loop and make a streak plate from the fluids in the bottom of the culture dish. (You should have practiced this technique.)

2. Invert plate and place in incubator for 24 hours.

3. After the streak plate has incubated for 24 hrs., observe and describe one isolated colony. Remember a single colony should be composed of just one type of organism.

4. Set up a water bath and melt tube of agar. Allow tube to cool to 45° - 50°C. This temperature must be maintained until the next step is completed.

5. Pick from the isolated colony you have chosen and mix into the melted agar. Twirl the tube between your palms to mix thoroughly. Re-flame the transfer loop or needle and carefully pick again, mix into agar, and twirl.

6. Pour the mixed melted agar tube into the sterile petri dish and allow to solidify. Set this dish aside for now.

7. Now you will pick again from the same colony with your flamed loop or needle and perform a Gram's stain test. (Refer back to the section on Gram's staining.)

8. Return to the pour plate which has now solidified and prepare your anti-microbial plate. With sterile forceps dip one of the absorbent paper discs into one of the antibiotics or disinfectants. Shake gently to remove excess fluid and press gently into the agar in one of the quadrants of the plate.

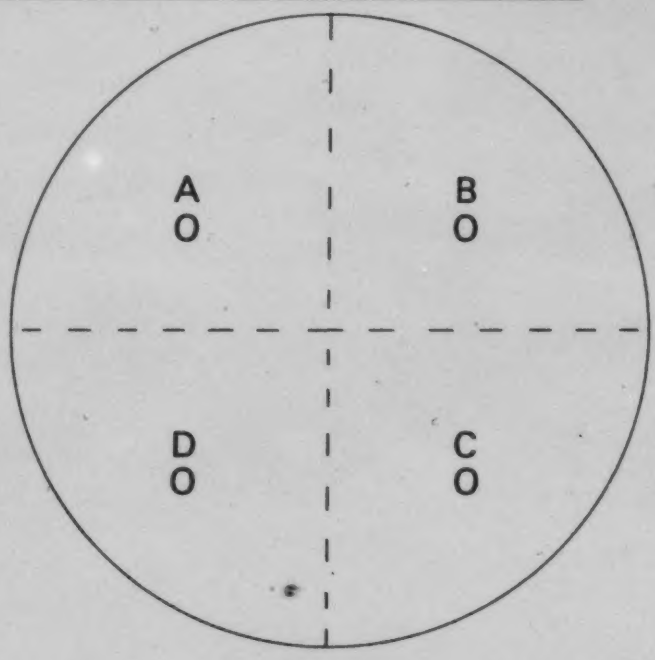
9. After 24 hrs. (48 in some cases) observe your antimicrobial plates. Measure the clear area around the disc and record on the data form with the rest of your findings.

After you have completed your data sheet, study it carefully and see how much knowledge you have gained about your unknown. You may not be able to specifically identify the particular organism at this point, and we didn't intend that you should.

But you should have gotten the "feel" of how the search is carried out in the clinical laboratory situation.

RECORD FORM

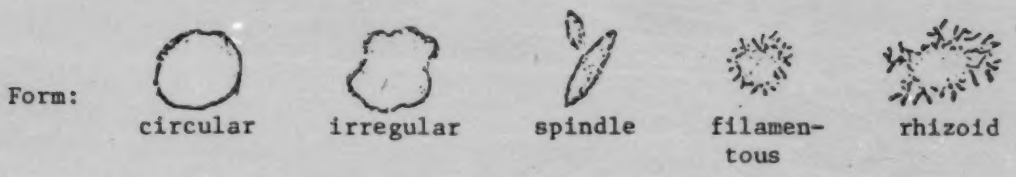
TRACKING AN UNKNOWN

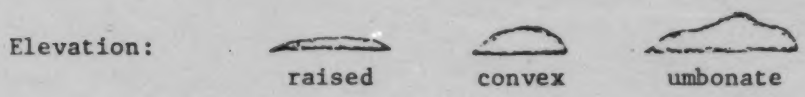
Drawing of a few cells as viewed during gram's stain	
magnification	
Description of cell morphology	
Results of Gram's stain	
Relative anti-microbial action of disinfectants A. B. C. D.	

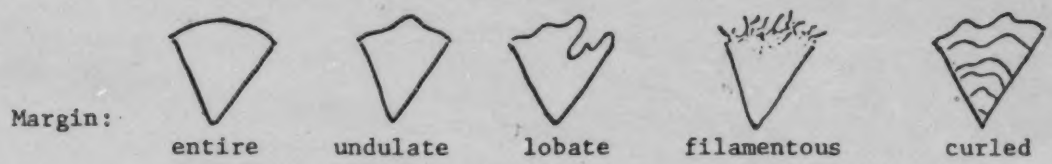
Shade with pencil to show relative size of clear areas.

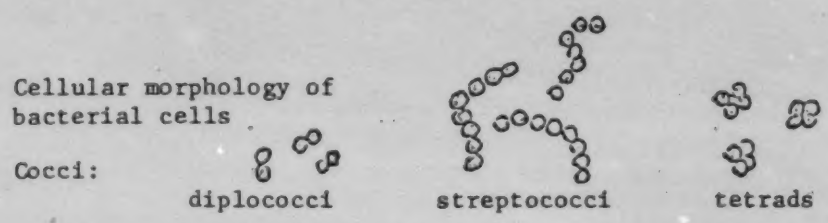
Appendix A

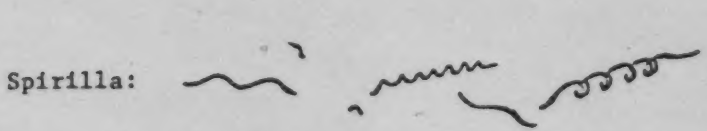
Culture Characteristics and Cell Morphology
culture characteristics of bacteria colonies









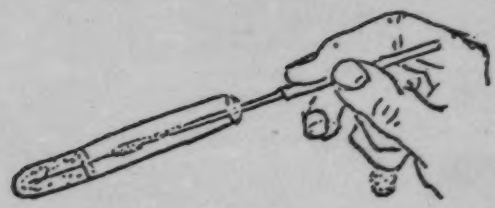


(after pelezar)

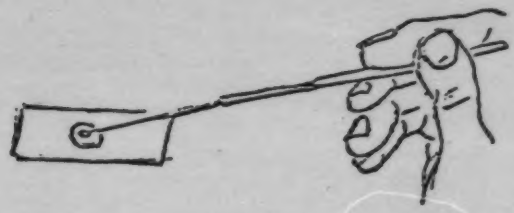
*most common form of bacillus

Appendix B

Equipment and Technique

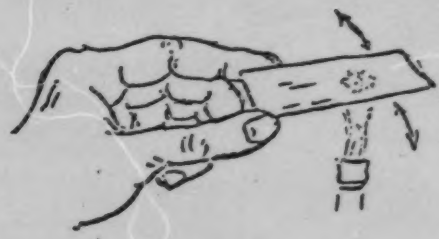


a. Specimen removed with Transfer Loop.

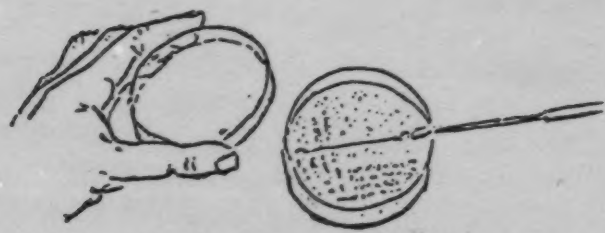


b. Material on transfer loop smeared over an area approximately the size of a dime.

c. The smear is "fixed" by passing the slide (smear side up) over the burner flame. Avoid excessive heat.



d. The transfer needle is sterilized by holding it in the flame of the burner.



e. The lid of the petri dish is raised. The loop is moved back and forth over the surface of the agar to distribute the organisms.

Appendix C

Glossary

aerobes -- those organisms needing gaseous oxygen for their growth

agar -- a complex carbohydrate obtained from brown algae and used as a base for many nutrient media. It is solid at room temperature and liquid at higher temperatures

anaerobes -- those organisms that can grow in the absence of gaseous oxygen

antibiotics -- naturally occurring materials which inhibit growth

aseptic -- characterized by the absence of microbial contamination

autoclave -- an instrument used to sterilize by means of elevated temperatures and steam under pressure

binary fission -- splitting into two cells, as in the case of bacteria

coccus (pl. cocci) -- spherical-shaped bacteria

colony -- a group of single-celled organisms

condenser -- a system of lenses used to focus the light from a microscope lamp into the objective of the microscope

culture -- to raise or foster the growth of an organism

filamentous -- threadlike

flagellum -- a hairlike object attached to certain protistans and used for propelling the organism

generation -- the whole group of organisms living at a particular time

generation time -- the time necessary for a population to double

Gram-negative -- bacteria that fail to take the Gram stain

Gram-positive -- bacteria from which the Gram stain cannot be washed out by alcohol

incubate -- to maintain organisms under conditions that will permit their growth

inoculum -- the starting source of organisms from which a culture develops

medium (pl. media) -- any number of solid or liquid materials upon which microorganisms grow

micro -- a prefix meaning small

microbe -- microscopic living being

mordant -- a substance that forms an insoluble combination with a dye, thereby making the color more permanent

morphology -- the study of form and structure

nutrient -- a food material

organism -- any living being

pathogens -- disease-producing organisms

physiology -- the study of function

sterile -- characterized by the absence of all living things

unicellular -- single-celled

UNIT V

LABORATORY EXERCISE

Paper Chromatography: an Analytical Tool

Paper Chromatography as an Analytical Tool

INTRODUCTION

More and more in recent years the battle against disease and physical impairment has required doctors and research scientists to identify various organic compounds. This is especially true in the field of protein chemistry. It has been known for a long time that most of our body organs, tissues, and cells are composed of very complex protein compounds. These compounds in turn are composed of a number of 'building blocks' called amino acids. There are about 20 different amino acids known to man.

$$AA_1 + AA_2 + AA_3 + AA_2 + AA_4 \text{ etc.} \longrightarrow AA_1 - AA_2 - AA_3 - AA_2 - AA_4 - \text{etc.}$$

amino acids → proteins

The proteins formed in this manner are very specific as for a number, sequence, and arrangement of its amino acids are concerned. A medium size protein molecule may contain thousands of amino acids. If just one of these is missing or out of sequence, the protein could give us an abnormal condition.

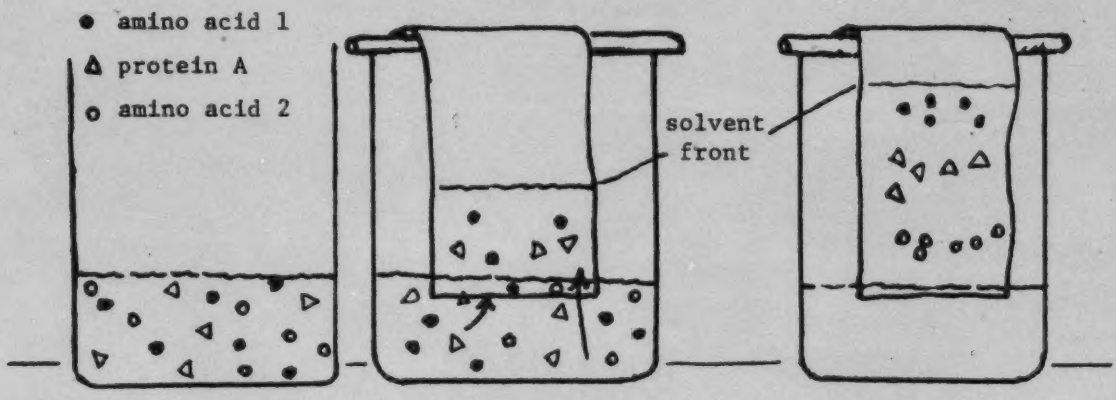
This was found to be the case in the dread disease, Sickle cell anemia. This disease is actually due to abnormal hemoglobin, the important oxygen carrying molecules found in human red blood cells. Human life depends on these molecules and their ability to carry oxygen to all the cells and tissues of our body.

The protein portion of the hemoglobin molecule is composed of approximately 588 amino acids arranged in a definite pattern. Through a process of isolating and determining the sequence of the amino acids it was found that in two sites on the molecule the amino acids on the 'diseased' hemoglobin differed from that of 'normal' hemoglobin. More specifically, the amino acid valine has replaced the amino acid glutamic acid at two locations on this very complex molecule.

Kind of Hemoglobin	Amino acids numbered in order							
	1	2	3	4	5	6	7	8
normal	valine	histidine	leucine	theronine	proline	*glutamic acid	glutamic acid	lysine
sickled	valine	histidine	leucine	theronine	proline	*valine	glutamic acid	lysine

Results of analysis by chromatography of the amino acid sequence in the segment of the hemoglobin molecule where valine(6) replaces glutamic acid(6).

The technique of paper chromatography, which played an important role in the above discovery, is basically of simple process. After the hemoglobin molecules were broken into smaller fragments of protein and amino acids, they were dissolved into a solution. Absorbent filter paper was dipped into this solution which moved along the paper by absorbtion. The dissolved proteins and amino acids moved along the paper with the solution but not all at the same rate.



solution of proteins & AA moving solvent front proteins & amino acids separated

Due to slight differences in their chemical make-up the various proteins and amino acids had a different attraction for the solvent as well as for the filter paper. Thus this caused their separation as the solvent front moved along the paper.

It was through a series of chromatography experiments like this that the biochemist learned which amino acids made up which compounds and also the sequence in which they appeared.

Objective:

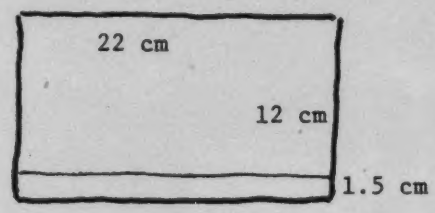
To acquaint the student with the concept of chromatography as an analytical tool. To construct a visible "fingerprint" identification of several amino acids. To identify an 'unknown' amino acid(s) by way of chromatography.

Materials:

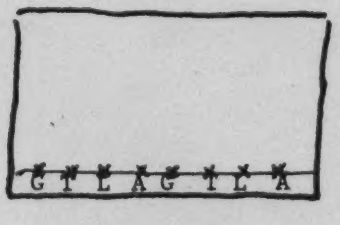
1000 ml beaker; aluminum foil; capillary tubes*; solvent; amino acids: glycine, tyrosine, leucine, aspartic acid, unknown; filter paper, ninhydrin spray, stapler, 4-inch test tubes.

Procedure: Part A

1. Obtain from your instructor a clean sheet of Whatman #1 filter paper*, about 12 cm by 22 cm, and make a light pencil line parallel to the bottom about 1.5 cm up.



2. Along this line, at intervals of about 2.5 cm, place eight x's. Under each x identify the amino acids, two for each acid.

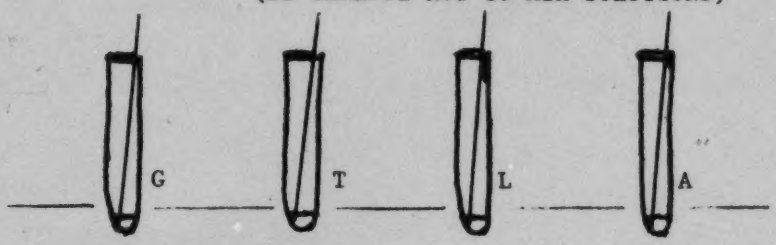


3. Place labels(amino acids) on 4 clean, 4-inch test tubes and place one capillary into each tube. From the stock solutions transfer a few drops of the appropriate amino acids into each of the

*Handle the paper as little as possible and only at the edges.

four test tubes.

(BE CAREFUL NOT TO MIX SOLUTIONS)



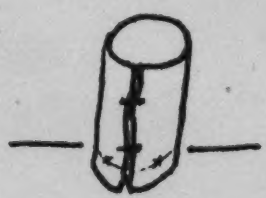
Your instructor will determine how many students are to work from each set of tubes.

4. Using the capillary tubes, place a small drop of each solution (amino acid) on its two positions along the line on the filter paper.

   a. avoid getting the spot on the paper larger than about 2 mm in diameter.
   b. it is advisable first to practice transferring solution to an ordinary piece of filter paper.

5. Let the paper dry for a few minutes and then add a second drop of solution at each spot.

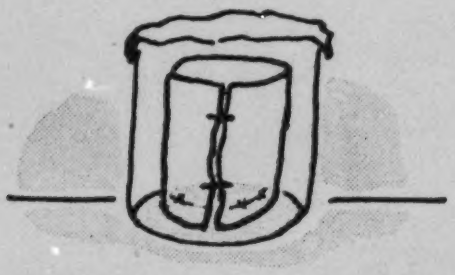
6. Roll the paper into a cylindrical form (watch your fingers), and staple the ends together about a third of the way from the edge. Staple so that ends of the paper do not touch each other.



7. While your spots are drying prepare the 1000 ml beaker by adding about 10cc of the solvent (use funnel to avoid contact with sides of beaker) and covering tightly with aluminum foil.

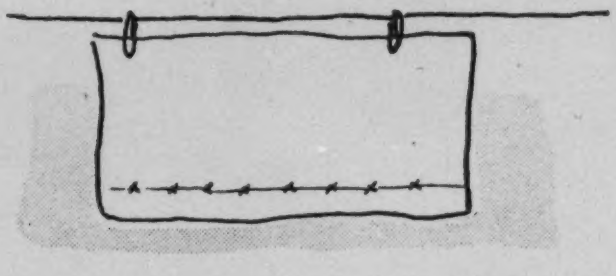
8. When spots are perfectly dry, place the cylinder carefully in the beaker of solvent and cover carefully and tightly with the foil. (cont'd.)

(Make sure the paper does not touch the sides of the beaker).



*See below.

9. Let the solvent rise up the paper about 3/4 of the way, about 7 or 8 cm. Remove the paper and place it upside down on your desk top to dry. Mark the solvent front lightly with pencil. When most of solvent has evaporated, open the cylinder by tearing it apart at the staples and hang it up to dry.



10. After drying, spray the paper lightly with ninhydrin and leave until the spray solution is dry.

11. Place the paper in an oven at 100 - 100°F for about 10 min. - or until the spots have developed.

12. Circle each spot with pencil and measure the distances each spot travelled (cm). Use the center of the spot for measuring.

13. Measure the distance the solvent travelled at each position and calculate the Rf values for each amino acid. (see following page.)

*If time does not allow completion of this step, your instructor will see to it that the cylinder is removed at the proper time and placed aside to dry. You can continue from step #9 tomorrow.

"Paper Chromatography as an Analytical Tool"

name_____

Data Chart
Rf values amino acids and unknown

Amino acids	Distance spot travelled(cm)	distance solvent travelled(cm)	Rf value	Approximate diameter of spot	color (if present)
Glycine					
Tyrosine					
Leucine					
Aspartic acid					
Unknown					

Identity of unknown_____

Questions:

1. What is the function of the ninhydrin spray in this exercise?

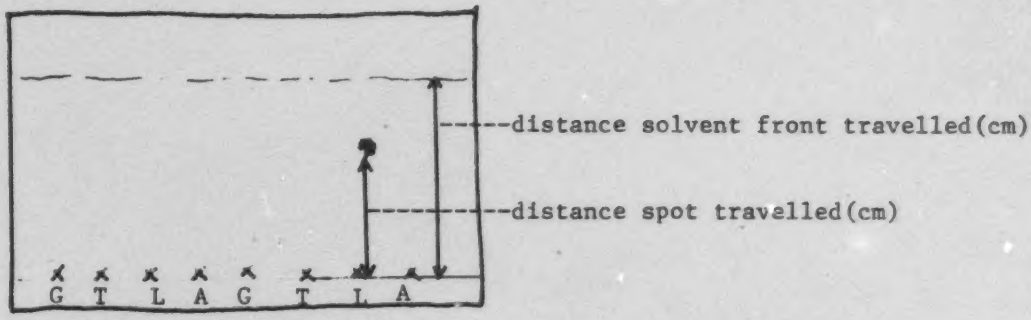
2. If there are only(approx.) 20 amino acids known to man, and many proteins contain all 20, how do you account for the fact that of the thousands of proteins that compose the biological world no two are alike?

3. What is the function of the hemoglobin molecules in the blood?

Questions (cont'd.)

4. Why is it recommended that you draw your lines and make your x's on the filter paper with pencil?

Diagram for step 13.



$$Rf = \frac{\text{distance spot travelled(cm)}}{\text{distance solvent moves(cm)}}$$

14. Enter findings on data recording sheet

Part B: Determination of Unknown

Procedure:

1. Set up filter paper as above but just place 3 x's on the pencil line.

2. Obtain an unknown from your instructor and proceed as from step 4 in Part A.

3. Enter findings on data recording sheet.

Related Vocational Fields

LIFE SCIENTISTS

Life scientists study living organisms, their structure, development, behavior and life processes. He usually specializes in one of three broad areas - agriculture, biology, or medicine. Two-fifths of all life scientists are engaged in research and developments. Thus they must be familiar with fundamental research techniques and use of light and electron microscopes and other complex laboratory equipment. Advanced techniques and principles from chemistry and physics are applied widely. Life scientists may be classified into three broad groups depending on the type of organism with which they work: Botanists, who study plants; Zoologists, who are concerned with animals; and microbiologists who work with microorganisms.

BIOCHEMISTS

The biochemist has an important role in modern science's search for the basis of life and the factors that sustain it. They study the chemical composition of living organisms. They analyze chemical compounds such as protein, minerals and sugars.

Foremost among the areas of application of biochemistry are medicine, nutrition, and agriculture. In the medical field, biochemists may investigate the causes and cures of disease or develop diagnostic procedures

There are many technician and technologist positions which require less training than that of the Life Scientists and the Biochemists.

UNIT VI

THE TRANSPORT SYSTEM

THE TRANSPORT SYSTEM

INTRODUCTION

In the modern medical laboratory a wide variety of analyses ranging from simple to complex are utilized by the physician and are essential to him in the care of his patients. Laboratory personnel, to qualify for such work, require specific training and education. Usually the laboratory is under the direction of a medical doctor, the pathologist, who specializes in the study of disease (pathology).

Under the direction of the pathologist is the medical technologist, who is educated to teach and perform the complex laboratory procedures, and to handle supervisory duties. To assist the medical technologist, the medical laboratory technician is specifically trained to perform routine laboratory tasks.

It takes a special kind of person to work in a clinical laboratory. He must understand the need for accuracy, be conscientious, and above all the technician should always be aware that his work will directly effect the health and welfare of other human beings.

The tenth grade units designed for the TACT Program are intended to impart some of the flavor of the various facets of Allied Health. It is hoped that by way of these experiences the student will become more aware of community health and what it may have to offer in terms of possible career choices.

LABORATORY EXERCISE

PART I. HEART RATE AND BLOOD PRESSURE

OBJECT: This lab exercise is designed to familiarize the student with the instruments and techniques which are employed during physical examination of the heart and vessels.

MATERIALS AND EQUIPMENT:

Stethoscopes (preferably Bowle's type), sphygmomanometers, alcohol, cotton, recording sheets

PROCEDURES:

SECTION A. Pulse rate

1. The member of the team serving as the 'patient' will be seated with his left arm in a relaxed position.

2. You will place your three middle fingertips over the radial artery in the wrist at the base of the thumb. (Do not use the end of your fingers for they might poke or scratch the 'patient'). If you have trouble locating radial artery, call instructor.

3. Count each beat for one full minute. Determine the rate by use of your watch or by the classroom clock. (If you are not sure of the count, repeat the process.)

4. You or your recorder will enter the pulse rate in the proper space: Pulse Rate-Quiet.

5. Have the patient exercise violently for about 40 seconds (jumping on one foot, push-ups, running upstairs, etc.) then take the pulse again. Record: Pulse Rate-Exercise.

6. Now you will play the role of the 'patient' while #1 is resting. But you must check the pulse of #1 again aproximately 2 minutes after completion of step #5 to see if his pulse has returned to normal. You will continue this until you have determined his recovery time. Record.

SECTION B. Heart Rate and Sounds

1. Be sure that the ear piece of the stethoscope has been thoroughly cleaned and that the 'patient' is in a relaxed position.

2. Insert the ear pieces and place the diaphragm on the lower area of the left chest just to the left of center. Move the diaphragm around until you pick up the beat.

3. Count the beat for 30 seconds (then multiply by 2) in order to determine beats/per minute. Do this several times and record the average reading in the box marked: Heart Rate-Quiet.

4. Have the patient exercise for about 40 seconds and immediately determine heart rate again. Record under: Heart Rate-Exercise.

5. Listen again for the heart sound. Record whether you think it is: strong and regular, dull and erratic, or any other combination of strength and rhythm you may have observed.

6. Remove the ear pieces, clean them with alcohol, and reverse your roles.

SECTION C. Blood Pressure

1. Be sure that the 'patient' has rested sufficiently to be back to normal. Seat the patient so that his left arm is resting on the table palm upward.

2. Place the sphygmomanometer inflatable cuff snugly (not tight) around the patients upper arm (as demonstrated).

3. Now with your stethoscope in place over the brachial artery and the B/P cuff valve closed proceed to pump air into the cuff until the indicator reads about 170 mm/Hg (or 170 PSI).

4. Now gently open the needle valve and allow the pressure to drop slowly (aprox. 3 mm or P.S.I. per second). You must keep your eye on the indicator dial and listen carefully for the initial sound (Systolic Beat). Rember the point at which this systolic beat was first heard!

5. Allow the pressure to continue falling, listening closely for the next characteristic sound (diastolic beat). Mark on your mind this second point and allow the cuff to completely deflate.

   Record your findings on the chart: B/P Systolic-Diastolic-Quiet

6. If you need help, call your instructor. You should try for a second reading if you weren't sure of the first readings. DO NOT TAKE THE PRESSURE OF ANY ONE PERSON MORE THAN THREE TIMES IN SUCCESSION!

7. You may reverse roles at this time and repeat the above steps.

8. Determine B/P after exercise as described above and enter readings for each 'patient' in the proper space.

   Be sure that the stethoscope is cleaned thoroughly and the sphygmomanometer is stored away properly.

PART II. EXAMINATION OF THE BLOOD: BLOOD SMEAR
WHITE BLOOD CELL COUNT
W B C DIFFERENTIAL

INTRODUCTION:

The preparation of a blood film is part of the routine blood tests done on most new hospital patients. The blood film (slide) is the only permanent record of blood work done on a patient which can be retained in the laboratory. The blood film has many uses. It is used to study the structural features of both the red cells and the white cells. It can also be used to verify the hemoglobin value and the red cell count as well as the white cell count and platelet count. Because the blood film is used for so many purposes, a well made smear is essential.

You will be required to make several blood films by which you can examine your own blood. The proper technique(s) will be demonstrated in class.

OBJECTIVE: This lab exercise is designed to familiarize the student with one of the techniques employed by the lab technician in the study of a patient's blood. The student will be able to compare the normal blood slide with several abnormal (pathogenic) slides.

MATERIALS AND EQUIPMENT:

Clean glass slides, alcohol (70%), gauze pads, Wright's stain, distilled H_2O, microscope with oil immersion lens, immersion oil, sterile blood lancets, phosphate buffer (6.4-6.8), staining racks or trays.

PROCEDURE: A detailed discussion and demonstration will be carried out by your instructor before you perform the following steps.

1. Study the prepared Wright's stain blood slides under <u>oil</u> immersion (You should have a text, blood atlas, duplicated handouts, or a wall chart to aid you in identifying the various blood cell types).

   a. Observe the Red Cells (ery throcytes).

      What color do they appear to be? _____

Sketch, carefully a small section of your microscope field to show the arrangement, shape, and comparative size of these cells.

Do these cells have a nucleus? _____

b. Observe the white cells (leucocytes).
(at this point you will have to refer to your references in order to identify the various types of white blood cells)

1. Neutrophils - these are the most common and compose 60-75% of the white cells.

Sketch carefully at least two neutrophils aprox. 3/4 inch in diameter showing as much detail as possible. Include in your sketch several red cells in order to show the comparative sizes.

2. Lymphocytes - these are the second most common white cells and occur about 20-30% of the total white cell count. (Read the description carefully)

Diagram several Lympocytes below aprox, 3/4 inch in diameter. Be as accurate as possible. Include several red cells in your sketch to show relative size.

3. Monocytes - occur aprox. 3-8% of total white cells. (Read the description carefully)

Diagram several Monocytes below as described above.

4. Eosinophils - occur aprox. 2-5% of total white cells. (Read description carefully).

Sketch several Eosinophils (Don't forget the red cells).

5. Basophils - occur about 0-1% of the total. (Read description carefully). It is possible that the slide you have contains no Basophils, try a different slide.

Sketch several Basophils below. Again, include several red cells to show relative size.

Now that you and your instructor are satisfied that you are adequately familiar with the various blood cells found in a typical smear, you may proceed to perform a W B C (white blood count) on your own blood.

PROCEDURE FOR PREPARING, STAINING AND EXAMINATION OF BLOOD FILMS:

The equipment used for making blood films must be meticulously clean. Precleaned slides or slides cleaned with alcohol and wiped dry are to be used for best results.

1. Select the finger to be used and clean the tip with the alcohol pad. Allow finger to dry.

2. Have your partner or your instructor prick your finger with the steril laucet.

3. Squeeze your finger so that a small drop of blood forms. Wipe off this first drop because it contains foreign substances which prevent it from being good for study.

4. Squeeze your finger again and this time place the drop on your clean slide about 1 inch from the end.

5. With a second slide make the blood smear as previously demonstrated. This step should be performed <u>immediately</u> after the drop is placed on the slide.

6. Gently squeeze the pricked finger again and make a second smear.

7. Wave both slides in the air to dry.

8. Label the film slide by writing your name in pencil in the blood at the thick end.

NOW YOU ARE READY FOR STAINING

PROCEDURE:

1. Fix the film by flooding the slide with Wright's stain for 3 to 5 minutes.

2. Add phosphate buffer using 1 to 1½ times as much buffer as stain on the slide. Add the buffer dropwise, and blow on the surface to mix the stain and buffer.

3. Allow this mixture to stand for 10 to 15 minutes.

4. Wash the slide with distilled water and pour off the staining solution at the same time. Remove all excess stain with this process.

5. Wipe the stain from the back of slide and allow the slide to drain in a vertical position with the heaviest part of the smear downward.

6. When dry, the slides are ready for microscopic examination.

EXAMINATION OF THE BLOOD FILM

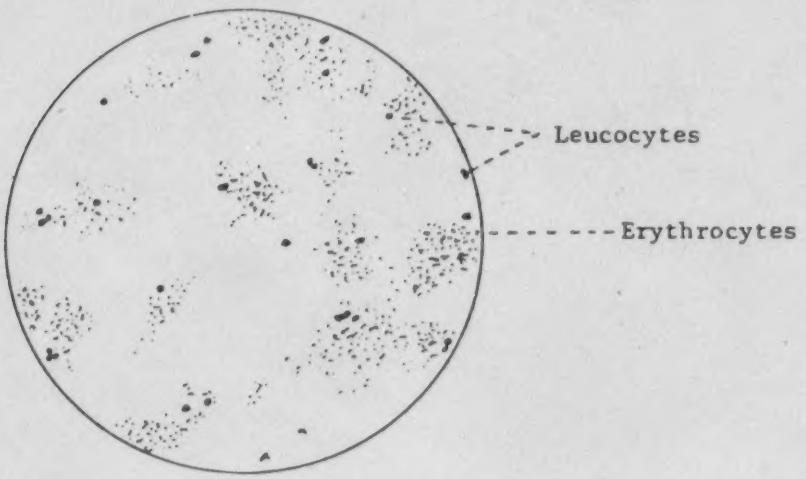
PROCEDURE:

1. Scan the slide under low power and locate an area where the red blood cells are just touching and not piled on top of one another. This should be near the thin edge of the smear.

2. Apply immersion oil to this region of the slide and proceed to focus as demonstrated.

Estimation of White Blood Count (WBC)

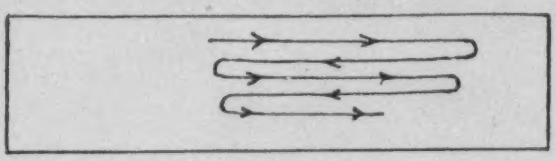
3. Using the low-power objective (10x) and the eye-piece (10x), with a total magnification of 100x, aproximately 20 to 30 white cells per field are equivalent to a white cell count of aproximately 5,000 cells/mm^3. With this same magnification, a count of 40 to 60 cells per field is equivalent to to a white cell count of aproximately 10,000 cells/mm^3. (The norm ranges from 7 to 9 thousand cells/mm^3)



Differential Count of White Cells

The differential count consists of identification and counting of a minimum of 100 white cells.

4. Move the slide to allow for a continuous count of the white blood cells. You should scan the slide from left to right or right to left, drop down aproximately the distance of one field and move slide in the opposite direction.



scanning pattern

5. No cells should be skipped you should attempt to identify and count all of the white cells. You will realize at this point that quick and accurate identification can only be obtained thru proper training and experience. But let's see how we make out.

6. When exactly 100 cells have been counted, the number of different white cells recorded is an estimate of the percentage of the various types. For example, when 3 of the 100 are identified as eosinophils, then they make up 3% of the total white cell population, etc.

   Keep a running tally:

example:	neutrophils	𝍸 𝍸 𝍸 𝍸 //
	lymphocytes	𝍸 𝍸 /
	monocytes	////
	eosinophils	/
	basophils	/

How do your findings compare with the percentage norms?

PERFORMANCE OBJECTIVES

PART I. HEART RATE AND BLOOD PRESSURE

1. You should be able to define and/or describe what is meant by pulse.

2. On a diagram of the human body, be able to locate and name the five pressure (pulse) points on the body.

3. You should be able to explain in your own words the physical characteristic of the arteries which enable them to produce a 'pulse'.

4. You should be able to state the relationship which exists between apical (heart) rate and radial pulse rate.

5. Be able to list at least three things that would contribute to an increased heart rate.

6. You must be able to explain what the heart sounds (lubb-dub) are actually attributable to.

7. You must be able to show and/or list the steps, in order of occurance, necessary to take a blood pressure reading.

8. Be able to match the instrument(s) and the vessel or organ concerned with the related test performed.

9. Be able to list at least three causes of high blood pressure.

PERFORMANCE OBJECTIVES

PART II. EXAMINATION OF THE BLOOD

1. You should turn in, for instructors approval, one well made and labelled blood film slide.

2. You should be able to list the steps, in order of performance, necessary to; prepare a blood film; and stain a blood film.

3. You must be able to list at least three uses of a stained blood film by the laboratory technician.

4. If shown a normal blood cell either via projector or under the microscope, you should be able to identify that cell, e.g. neutrophil, lymphocyte, etc.

5. You should become familiar with, and be able to relate in terms of probable cause, symptoms, effect on blood cells, and possible treatment, of two of the more common diseases which lead to blood disorders. (You may have to visit the library to satisfy this requirement).

INDEX II: BLOOD CELL DATA SHEET

ERYTHROCYTES (RED CELLS):

35-45% of blood by volume
4.5-5.0 million/mm^3 (women)
5.0-5.5 million/mm^3 (men)

Normal erythrocytes are biconcave discs, 6 to 8 microns in diameter and 1.5 to 2.5 microns thick. In stained smears they appear as pink circular objects with distinct and smooth margins.

LEUCOCYTES (WHITE CELLS):

7.0 to 9.0 thousand/mm^3

A. Granular Leukocytes (formed in bone marrow)

1. Neutrophils: 60-75%, 10-12 microns in diameter; nucleus deep purple with 3-5 lobes; cytoplasm pink and finely granular

2. Eosinophils: 2-5%; about 12 microns in diameter; nucleus light blue with 2 lobes; pink cytoplasm with many large uniform granules stained bright red with Wright's Stain.

3. Basophils: 0.0-1.0%; 10-12 microns in diameter; irregular non-segmented dark blue nucleus; scattered large irregular granules stained blue-black with Wrights Stain; cytoplasm light blue.

B. Agranular Leukocytes (formed in lymphoid tissue)

1. Lymphocytes: 20-35%; about 10 microns in diameter; large round or indented nucleus stained purple; scanty to pleantiful cytoplasm stained sky-blue with Wright's stain; few irregular dark red granules may be present.

2. Monocytes: 3-8%; 12-20 microns in diameter; nucleus red purple, irregular, and lobes; pleantiful cytoplasm stains dull grayish blue, often frothy looking, sometimes contains vacuoles; few pin-point granules may be present.

3. Platelets: 150-300,000/mm^3; 2 to 4 microns in diameter (note size as compared to the crythrocytes) these cell fragments are found dispersed among the red cells and you have to look hard to be aware of them; tendancy to clump; colorless to pale blue background; reddish to violet granules.

UNIT VII

INDIVIDUAL STUDY

a report on a

COMMUNICABLE OR HEREDITARY DISEASE

INTRODUCTION

It is hoped that the writing of this paper does not become a burdensome chore. But rather it proves an interesting and challenging task which will allow you to take an in-depth look into an area of community health which may hold some interest for you.

The accompanying list of communicable and inheritable diseases are listed for your convience and you are by no means limited to these suggestions. It is hoped that by creating a broad number of possible topics not more than two, three, or four students will be writing in the same area.

The outline presented below is intended to help you present your information in an interesting and readable form. The various headings should be emphasized by capitol letters, indentation, or skipping several lines in order to make it easier for the reader to follow the chain of thought. That is, if a person wishes to learn about the means of transmission of Typhoid Fever, he can readily locate that section of your paper by the obvious sub-heading.

Independent Study

Communicable and Inherited Diseases

Student may choose from:

1. Arthritis
2. Bronchitis
3. Chicken Pox
4. Color Blindness
5. Cretinism
6. Diabetes
7. Diptheria
8. Epidemic Encephalitis
9. Epilepsy
10. Berman Neasles or Rubella
11. Hemophilia
12. Impetigo (skin)
13. Infectious Hepatitis
14. Influenza
15. Leukemia
16. Mononucleosis
17. Multiple Sclerosis
18. Paget's Disease (bone)
19. Pneumonia
20. Poliomyelitis
21. Q Fever
22. Scarlet Fever
23. Sickle Cell Anemia
24. Staphylococcus infections
25. The common Cold
26. Trachoma (blindness)
27. Tuberculosis
28. Typhoid Fever
29. Venereal Disease
30. Small Pox

Areas:

1. History
   Body systems,organs, etc.
2. Symptoms
3. Therapy
4. Immunity
5. Means of Transmission
6. Community Medical Resources
7. Research in field
8. Interview
9. Readings cited

OUTLINE

Suggested arrangement for the presentation of the body of your report

1. Name(s) of the disease

2. History
   a. When and where the disease was discovered
   b. the men whose names are connected with investigation of this disease
   c. any particular geographical location where the disease is most prevalent
   d. age range or ethnic group most susceptible

3. Symptoms
   a. how detected (fever, sores, swelling, etc.)
   b. body organs or systmes involved

4. Therapy
   a. relate means of treatment
      1. drugs, radiation, surgery, etc.
   b. immunity
      1. is there natural immunity
      2. is there acquired immunity
   c. length of time of treatment
   d. mortality rate

5. Means of transmission (how contacted?)
   a. bacterial or viral
      1. air borne
      2. water borne
      3. physical contact
      4. intermediate host
   b. congenital
   c. hereditary

*6. Community medical resources
   a. health care service mainly involved with disease?
      1. dentists, surgery, optometry, psychiatry, etc.
   b. sub-services which might be involved?
      1. inhalation therapy, physical therapy, dietetics, speech therapy, etc.
   c. what role do the above personnel perform?

7. Present research or work being conducted
   a. This sort of information might best be found in health and medical journals.

8. Readings cited
   a. This is not a bibliography, you need only list those resources that you actually used to prepare your paper.

Outline (Cont'd)

9. Glossary
   a. You should list and define, in alphebetical order, all terms in your paper which you think might be difficult for the average reader to understand.

* This area might best be approached by way of an interview with your family doctor or any other qualified member of the community health team.

U.S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE
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ALLIED HEALTH FIELD

TENTH GRADE

INTRODUCTION TO ALLIED HEALTH AND THE HEALTH CARE TEAM

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Operation TACT

Curriculum

Teachers' Handbook

ED071158

TENTH GRADE CURRICULUM

Operation TACT

TEACHER'S GUIDE

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THE MICROSCOPE
(an analytical tool)

Introduction:

This unit, THE MICROSCOPE, is intended to be used after the students' general introduction to the microscope and the cell. It is designed to impart a practical aspect to the usual microscope labs. To extend the use of the microscope into the field of community health and to allow the student to experience a feeling of discovery will generate a more meaningful learning situation.

The slides mentioned in the text are suggested examples and are by no means all inclusive. It is hoped that the instructor will substitute others which he feels are more appropriate or more convenient to come by.

Resources:

This unit offers a wonderful opportunity for the student to become aware of the many and varied job opportunities related to the clinical laboratories department. A visit by a pathologist, histologist, etc., would be a fitting extension of this unit. You could arrange with the resource person to present his own collection of slides and relate the steps and techniques which are involved in the study of suspected diseased tissue. Arrangements would have to be made beforehand and the TACT staff would be more than willing to assist in this respect.

If the class interest seems to lean in the direction of Sickle Cell Anemia, the Connecticut State Department of Health has a team who travel around the state to educate people about this disease.

Procedure:

1. The activity itself is fairly cut and dry. It will be up to the instructor to familiarize the students with the characteristics of the "normal" tissue. A brief discussion of the disease and/or the disease-causing organism should be given before the student is given the "diseased" slide.

2. Sickle Cell Anemia - a brief but good description of the "abnormal" hemoglobin molecule and how its structure was evolved can be found in Ann Rollers - Discovering the Basis of Life and a more detailed account in one of the Scientific American offprints.

THE MICROSCOPE
(a diagnostic tool)

Behavioral Objectives

1. You should be able to match the six main fields involved in the hospital clinical department with the body system or area of investigation it is concerned with.

2. You should be able to identify and name the causative organism or disease connected with each of the pathologic slides used in this unit.

3. You should be able to define the following terms: intermediate host, parasite, pathology, tissue, hemoglobin, anemia,

4. You should be able to point out on the instrument or label a diagram of the student compound microscope and give the function of each part (where possible).

References:

Bushbaum, Animals Without Backbones

Moon, Modern Biology

Elliot, Zoology

Hickman, Integrated Principles of Zoology

Films:

Rival World - Shell Oil Co.

Microbes and Man

Part 1 - The Simple Stain

Introduction:

Strictly speaking, a dye may be any colored substance that, when combined with a second substance, imparts a color to that substance. However, most of the dyes used in microbiology are organic compounds, chiefly benzene derivatives.

Most microbiological dyes may be thought of as one or the other of two types of salts:

1) acidic, those in which the color bearing ion, the chromophore, is the anion; e.g., sodium + eosinate (the dry eosin); and
2) basic, in which the chromophore is the cation; e.g., methylene blue + chloride (the dye methylene blue).

In general, acidic dyes combine more strongly with cytoplasmic (basic) elements of cells. Bacteria are best stained with basic dyes.

The dyes used in this section: methylene blue, safranin, crystal violet, basic fuchsin, and eosin, are considered to be basic dyes which belong to the group of aniline (coal tar) dyes.

Materials:

1. The organisms used in Part 1 - the Simple Stain can be "homemade" by tearing up some lettuce leaves, using cream cheese, lima beans, pepper pods, etc. Just put this material into individual culture dishes with distilled H_2O and put away for a couple of days in a warm, dark place. You will get a mixture of organisms with this method and their exact identity is doubtful. But they will serve the purpose for a simple stain and the students will see the result of organic material decaying or rotting.

   Specific organisms purchased from a supply house and streaked on agar or grown in broth does have its advantages. In this case you will know the exact identity of the organism and be able to use them for Part II, The Gram Stain.

Procedures:

1. The techniques needed here can be practiced in "dry runs" until the student is familiar with the steps.

Findings:

Word description of bacterial structure and description of colonies should be referred to Appendix A at the end of this unit.

Part II - The Gram Stain

Background:

Mechanism of the Gram Stain. Although still the subject of some debate, it now appears that the property of the gram positiveness depends upon the presence and the physiochemical nature of the cell wall of gram-positive organisms. It has been shown that any disruption of the physical or chemical integrity of the cell wall or other parts of the cell results in gram-negativeness.

Evidence indicates that the crystal violet and iodine form a compound or precipitate inside the wall and cannot readily pass out in the presence of the decolorizing agent. In the opposite respect, gram negative organisms cannot retain the dye-iodine complex in presence of the decolorizing agent.

It is important to note that some organisms are "borderline" cases in respect to Gram's stain, sometimes being positive, other times negative. Both positive and negative cells of the same organism can be seen in the same culture.

Slight variations in culturing conditions and/or staining techniques can cause such results; e.g., age of organisms, pH of cultural fluid, or culture medium.

Whatever the explanation of the Gram reaction, it is important to note that there are characteristic differences between most gram-positive and gram negative bacteria. Several of the most obvious differences are shown in the following table.

WATER SAMPLING - Samples of water should be taken directly from the source under study, using sterile glass bottles with tightly fitting tops or stoppers.

If you have to provide your own sterile bottles, sterilization can be accomplished by heating in your oven at 400° F for about 30 min. The collecting bottle should be capable of holding from 300 - 500 ml (a little more than ½ pint).

In sampling from a lake, pond, or stream, the bottle should be placed below the surface and moved forward while being filled, so that the water coming in contact with the hands does not enter the bottle.

If there is a current, be sure to collect your sample moving the bottle upstream as you collect.

In shallow water be certain not to collect materials off of the bottom.

The sample should be returned to the laboratory as soon as possible for processing, preferably within 6 to 12 hours, since there will be changes in number of microbes during storage.

If the sample must be stored for any length of time, it should be kept cool. Your refrigerator can be used for this purpose.

Temperature of Sample - Air temperature at the site of collection and temperature of the sample at the time of collection should be recorded. The temperature should be read to the nearest .5° centigrade.

The thermometer used will probably have to be provided by your instructor. The thermometer is made of glass so need I tell you exercise the greatest of care when handling. The thermometer will be signed out to your team and you are responsible for it.

Physical Nature of Collection Point - You must be able to pin-point your collection point with accuracy. Your recording sheet contains some of the physical features you are to be aware of. Look this over carefully beforehand.

But a carefully written description by you will also be necessary. Was the sample taken from an inlet; are there houses or factories nearby, how close; is the area rocky or sandy; is there much bio-degradeable debris present, of what type, etc. All of these factors may play an important role in the nature of your collected samples.

Procedure:

1. Bacillus subtilis - gram positive
   Escherichia coli - gram negative

2. You might want the students to perform a Gram's Stain on some scrapings from the base of their teeth and gums.

3. You should remind the students that these slide preparations are viewed without a cover slip and are apt to be messed up after one viewing because of the immersion oil. It is suggested that each student make more than one slide preparation. They can be labelled and put away for a future class period.

Part III Tracking an Unknown

You should take time at this point to review what has happened up to now and answer any questions that the students might have. This would be a good juncture to bring in the community health aspect involving the search for the identity of a microbe.

It is also important to set up a definite time schedule for the events in this section. The Tracking of an Unknown will take the better part of a school week, but can be modified to fit your schedule.

Procedures:

1. One streak plate for every two students should be satisfactory.

2. Inverting the plates prevents condensation from dripping down into the surface. Be sure agar has solidified before inverting.

4. Agar melts at 100 degrees C and remains liquid down to about 45 degrees C. A good point at which to innoculate the melted tube is when it is very warm while holding it in the palm of your hands but not so hot that you can't stand it.

5. This step must be carried out without the loss of time so that the tube will still be liquid for step #6. Twirl the tube, do not shake or bubbles will form and remain in your final product.

8. This plate has to be inverted also, so the student must be gentle in order not to loosen the antibiotic discs. (These discs can be prepared by using a paper punch on filter paper.)

Laboratory Exercises Microbes and Man

Part I Materials

petri dish (cultures previously prepared)
culture tube
transfer loop
transfer needle
forceps
dropper bottle
staining solutions
methylene blue
carbol fuchsin
crystal violet
transfer needle or loop
microscope slides
oil (for oil immersion)

Part II Cultures of B. subtilis and E. coli
staining reagents (crystal violet)
Gram's iodine
95% alcohol
safranin
clean slides
distilled H_2O
burner
absorbent paper discs

Part III nutrient agar plate
slides
Gram stains
agar tube
sterile petri dish
transfer loop
variety of disinfectants
absorbent paper discs
forceps

PAPER CHROMATOGRAPHY
(an Analytical Tool)

1. The solvent, as recommended in Selected Experiments in Organic Chemistry by Helkamp and Johnson, consists of a 2% ammonium hydroxide and stock isopropyl alcohol. They are in the ratio of 10 ml to 20 ml respectively.

   Each student or team will require approximately 10 ml of the solvent.

2. The stationary phase calls for Whatman #1 filter paper. Other acceptable chromatography paper can be used. (The filter paper should be handled as little as possible to prevent foreign substances from becoming a part of the chromatogram.)

3. The capillary tubes should be made from a piece of 6-8 mm soft glass tubing heated and drawn to about the diameter of a thin sewing needle. These capillaries should be much smaller than those usually used for determination of melting point. They should be about 5-6 inches long and one of the ends should be square. This can be accomplished by scratching the glass with a carborundum chip and bending away from the scratch.

4. The amino acid solutions should be at a concentration of about a 0.05 molar each, in 1.5% hydrochloric acid.

   For a stock solution of the amino acids which, barring accident, should be adequate for the experiment:

AA	Grams	1.5% HCl
glycine	0.10	27 ml
tyrosine	0.20	22 ml
leucine	0.20	30 ml
aspartic acid	0.20 g	30 ml

   The mixture should be dissolved thoroughly with a magnetic stirrer.

5. The ninhydrin spray comes in an aerosol can and can be purchased from most scientific supply houses. It is used as a chromatogenic agent to "develop" the almost invisible amino acid spots on the chromatograph.

   The reaction basically involves the NH_3 which is released from the amino acid and combines with the ninhydrin molecules to form a blue-colored compound. Caution: Ninhydrin should be kept off of the skin because it reacts with body protein to form a long lasting purple discoloration.

6. The Rf factor for a particular amino acid is variable depending upon the conditions of pH of the solvent, nature (make-up) of the solvent, temperature, paper, and time solvent is allowed to run. For any particular set of the above conditions your findings (Rf) will be consistent. This is the time to impress upon the student that much of his work is actually original. Probably no other derived data was arrived at under exactly the same experimental conditions. Consistency and accuracy of measurement are essential and the student must employ the same technique in each of his trial runs.

7. The unknown can be prepared by mixing any combinations of the "known" amino acids in equal proportions. Again every piece of glassware must be plainly labelled and the unknown with a clean capillary tube should be given out by the instructor.

8. The solvents should be kept in a tightly stoppered container in a cool place. If possible, they should be dispensed under a hood or in a well ventilated area.

BEHAVIORAL OBJECTIVES

CHROMATOGRAPHY

1. You should be able to define the terms: solvent, absorption, Rf factor, hemoglobin.

2. You must be able, without the aid of notes or text, to diagram the basic structural formula for an amino acid.

3. You should be able to explain verbally or in writing the theory behind the technique of paper chromatography.

4. Given any two of the valves, the student should be able to determine either the Rf factor, the distance solvent traveled, or the distance the spot traveled. Remember:

$$Rf = \frac{\text{dist. spot traveled}}{\text{dist. solvent traveled}}$$

5. You should be able to list the steps, in a logical sequence, necessary to determine the Rf factor of an amino acid.

References:

Stein and Moore: CHROMATOGRAPHY, Scientific American, March 1951.

Helmkamp and Johnson: SELECTED EXPERIMENTS IN ORGANIC CHEMISTRY, W. H. Freeman and Co., 1964, pp 138-140

Routh, Eyman, & Burton: ESSENTIALS OF GENERAL, ORGANIC AND BIOCHEMISTRY. W. B. Saunders Company, 1969, Ch. 27.

Perutz: THE HEMOGLOBIN MOLECULE, Scientific American, 1964.

Laboratory Exercise

Part I - Heart Rate and Blood Pressure

This exercise should be proceeded by a discussion of the role of the heart and the characteristics of the vessels. The symptoms, treatment, and mortality, may help to stimulate student interest and make him want to know more.

Equipment

It is hoped that there will be adequate materials to divide the class into teams of three's. Stethoscopes and sphygmomanometers can be obtained on a loan basis by contacting the TACT office.

Background

Section A: Pulse Rate

1. The left arm being closer to the heart usually gives a stronger pulse. It might be worthwhile at this juncture to have the students try both right and left to see if they notice any difference.

2. You might wish to relate to the student that you are actually pressing the brachial artery against the radius, thus the derivation of the term radial artery. To avoid possible embarrassment it might be well to inform the students that the radial artery is not always found at the same depth; on some it is shallow (superficial) while on others it is located in a much deeper position, thus harder to locate.

3. You might wish to shorten the length of time to 10 seconds or 30 seconds and have it repeated several times and take an average.

4. The pulse rate is usually a little higher than normal during such an exercise.

6. You will probably have to take time to explain this step. The same person should take the patient's pulse when determining recovery time.

Section B: Heart Rate and Sounds

2. A chart or diagram to show the relative location of the heart should be available.

5. Heart sounds being caused by the closing of the valves means that different areas of the heart produce different sounds. The student, after locating the heart, should move the scope around to discover this fact. What might sound like a murmur may just be due to the fact that the area being listened to has overlapping value sounds.

Section C: Blood Pressure

1. The arm should be as near to "heart high" as possible.

4. and 5. You will probably have to take time out to describe the characteristic sounds that student should be listening for. Stress the fact that room noises should be kept at an absolute minimum during this process.

PART II EXAMINATION OF THE BLOOD

Materials and Equipment

1. Complete blood smear kits are available from the Lab AIDS, Inc. which will save a lot of preparation time. (Their plastic slides, which are very thin, may prove inadequate with certain types of mechanical arms which hold slides in place on the stage.)

2. The phosphate buffer can be substituted for by distilled water of pH approximately 6.5.

3. An oil immersion lens is most essential in blood work and might prove to be a hang-up. The Bausch & Lomb O.I. lens sells for about 35 dollars for student microscopes. I am sure other makes are comparable. A call to the TACT Office might also help to solve this problem if it exists.

Procedure:

1. A step-by-step demonstration of the oil immersion technique should be given. It would probably help the student if he refrained from going from hi dry to oil immersion until you can be standing by.

   NOTE: The immersion oil which has a refractive index the same as glass (1.52), prevents many of the divergent peripheral rays, and those rays lost by reflection, and refraction at the surfaces of the condenser, slide, and objective lens from being uselessly scattered. Many of these rays will now be collected and concentrated up through the objective to the eye. Thus the objective can be brought much closer to the object (slide) with a resulting working distance of about 0.2 mm instead of 1.8 mm. Thus the resolving power is greatly increased.

   Caution: In cleaning the lens avoid using Fluid Solvents that can act upon the cement which may be used to hold the lens in place. Use only lens paper to wipe the lens.

References:

Laboratory Exercises in Microbiology, 2nd ed., Michael J. Pelczar McGraw-Hill Book Company.

Basic Laboratory Techniques for the Medical Laboratory Technician 1970, Linne-Ringsrud, McGraw-Hill, Inc.

Index II - Blood Cell Data

EXAMINATION OF WATER

Introduction:

Needless to say, the examination of surface water can become a very involved process. The student should realize from the beginning that this exercise is limited in scope by time, facilities, equipment, etc. At the same time, he should be made to feel the importance of obtaining data which will help to establish a profile of a certain body of water. Each body of water, regardless of how large or small, comprises its own unique environment.

The present concern with pollution and the fact that the students will be able to examine water in their own community should add an air of excitement to this unit. We should impress upon the student that we are not necessarily just looking for pollution; we are trying to gather and interpret data which will make us more cognizant of the natural world around us.

A copy of STANDARD METHODS FOR THE EXAMINATION OF WATER, ETC., 10th Ed., American Public Health Association, N.Y., is an indispensable reference for work of this type. A shorter, lab manual-type book, ENVIRONMENTS IN PROFILE by Kaill & Frey, 1973, Harper & Row Pub. Inc., contains many simplified methods of examination which might enable you to extend the unit in the future.

Displaying data:

A large wall map of the Hartford area can be obtained from the City Engineer's Office for little or no cost. Small numbered flags can be used to indicate the various areas where water was collected. A piece of colored yarn running from the point of collection to a 5 x 7 card off the map can offer a quick and effective means of presenting collected data. This might even be placed in a central location in the school accessible to the whole student body.

Procedure:

a. The sterile water dilution blanks should be prepared beforehand and stored in the refrigerator. If no autoclave is available, boiled distilled water will suffice.

   The test tubes and/or the 100 ml bottles can be sterilized in an oven at 400°F for one hour. Make certain that all glassware for filling the dilution blanks have been sterilized.

   Each student or team should have enough dilution blanks to make at least one small dilution (say 1:100) and one fairly large dilution (at least 1:100,000).

The student should be made aware of the fact that it is impossible to estimate the number and type of organisms present in any water sample just by sighting. The fact that one particular sample may have 1 million organisms/ml and a second less than 24/ml is not necessarily an indication of pollution in either case. Water temperature, salinity, organic matter, pH, size, depth, flow rate, source, etc. all play a role in determining the number of organisms.

b. If a constant temperature water bath is not available, a temperature controlled hot plate will adequately serve this purpose.

Each student or team should be provided with at least 2 nutrient agar tubes (one for each of his final dilutions). Extra tubes should be available for "mistakes" and for additional dilution ranges if necessary.

It will probably be to your advantage to purchase the sterile nutrient agar tubes from a commercial laboratory rather than attempting to make your own. Bacto-Nutrient Agar is recommended for examination of water by "Standard Methods" (see Difco Manual).

c. Important: The student must be ready to pour his "plate" immediately after adding the 1 ml of sample (diluted) to the melted agar (45° C) and mixing. The added one ml of sample will cool the melted tube of agar and there is the possibility that the mixture will solidify before you are able to pour the plate.

Remind the student that shaking the tube to mix the sample and agar will result in bubbles which will remain in the poured plate and give us poor results.

Explain to the student the reasons for inverting the petri dish before incubation.

Plastic petri dishes are acceptable and easily incinerated after use.

Fecal Pollution

Endo Agar and E.M.B. Agar are the two solid media specified by Standard Methods for the examination of water and sewage. These can be utilized the same as the nutrient agar in the preceding section.

Each student or team should have available at least two of the differential agar tubes.

Drinking water should show "no" coliform growth. Swimming water should be between 200-2000 organisms per ml.

Determination of coliform organisms in the sample does not necessarily mean that we have a pathological condition. It does, however, give us an indication that pathogens might be present and serve as reason for further tests.